```
=> varki a/au
L1            1 FILE AGRICOLA
L2          111 FILE BIOTECHNO
L3           25 FILE CONFSCI
L4            0 FILE HEALSAFE
L5           66 FILE LIFESCI
L6           48 FILE PASCAL

TOTAL FOR ALL FILES
L7          251 VARKI A/AU

=> NeuGc and NeuAc
L8            3 FILE AGRICOLA
L9           83 FILE BIOTECHNO
L10           1 FILE CONFSCI
L11           0 FILE HEALSAFE
L12          38 FILE LIFESCI
L13          21 FILE PASCAL

TOTAL FOR ALL FILES
L14         146 NEUGC AND NEUAC

=> l7 and l14
L15           0 FILE AGRICOLA
L16           0 FILE BIOTECHNO
L17           0 FILE CONFSCI
L18           0 FILE HEALSAFE
L19           0 FILE LIFESCI
L20           0 FILE PASCAL

TOTAL FOR ALL FILES
L21           0 L7 AND L14

=> neu5Ac and neu5Gc
L22          11 FILE AGRICOLA
L23          45 FILE BIOTECHNO
L24           0 FILE CONFSCI
L25           0 FILE HEALSAFE
L26          24 FILE LIFESCI
L27          17 FILE PASCAL

TOTAL FOR ALL FILES
L28          97 NEU5AC AND NEU5GC

=> l28 and substract
L29           0 FILE AGRICOLA
L30           0 FILE BIOTECHNO
L31           0 FILE CONFSCI
L32           0 FILE HEALSAFE
L33           0 FILE LIFESCI
L34           0 FILE PASCAL

TOTAL FOR ALL FILES
L35           0 L28 AND SUBSTRACT

=> l28 and (comparing or compared or comparison)
L36           1 FILE AGRICOLA
L37          10 FILE BIOTECHNO
L38           0 FILE CONFSCI
L39           0 FILE HEALSAFE
L40           7 FILE LIFESCI
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```
L41          4 FILE PASCAL

TOTAL FOR ALL FILES
L42         22 L28 AND (COMPARING OR COMPARED OR COMPARISON)

=> dup rem
ENTER L# LIST OR (END):l42
PROCESSING COMPLETED FOR L42
L43          15 DUP REM L42 (7 DUPLICATES REMOVED)

=> l43 and antibody
L44           1 S L43
L45           0 FILE AGRICOLA
L46          10 S L43
L47           1 FILE BIOTECHNO
L48           0 S L43
L49           0 FILE CONFSCI
L50           0 S L43
L51           0 FILE HEALSAFE
L52           2 S L43
L53           0 FILE LIFESCI
L54           2 S L43
L55           0 FILE PASCAL

TOTAL FOR ALL FILES
L56           1 L43 AND ANTIBODY

=> d l56 ibib abs total

L56   ANSWER 1 OF 1  BIOTECHNO  COPYRIGHT 2009 Elsevier Science B.V. on STN
ACCESSION NUMBER:        2001:32206403   BIOTECHNO
TITLE:                   Characterization of cytosolic sialidase from Chinese
                         hamster ovary cells - Part II. Substrate specificity
                         for gangliosides
AUTHOR:                  Muthing J.; Burg M.
CORPORATE SOURCE:        J. Muthing, Institute of Cell Culture Technology,
                         Faculty of Technology, University of Bielefeld, PO Box
                         100131, D-33501 Bielefeld, Germany.
                         E-mail: jm@zellkult.techfak.uni-bielefeld.de
SOURCE:                  Carbohydrate Research, (15 FEB 2001), 330/3 (347-356),
                         58 reference(s)
                         CODEN: CRBRAT  ISSN: 0008-6215
PUBLISHER ITEM IDENT.:   S0008621500002950
DOCUMENT TYPE:           Journal; Article
COUNTRY:                 United Kingdom
LANGUAGE:                English
SUMMARY LANGUAGE:        English
AN    2001:32206403   BIOTECHNO
AB    Cytosolic Chinese hamster ovary (CHO) cell sialidase has been cloned as a
      soluble glutathione S-transferase (GST)-sialidase fusion protein with an
      apparent molecular weight of 69 kD in Escherichia coli. The enzyme has
      then been produced in mg quantities at 25-L bioreactor scale and purified
      by one-step affinity chromatography on glutathione sepharose (Burg, M.;
      Muthing, J. Carbohydr. Res. 2001, 330, 335-346). The cloned sialidase was
      probed for desialylation of a wide spectrum of different types of
      gangliosides using a thin-layer chromatography (TLC) overlay kinetic
      assay. Different gangliosides were separated on silica gel precoated TLC
      plates, incubated with increasing concentrations of sialidase (50
      μU/mL up to 1.6 mU/mL) without detergents, and desialylated
      gangliosides were detected with specific anti-asialoganglioside
      antibodies. The enzyme exhibited almost identical hydrolysis
```

activity in degradation of G.sub.M.sub.3(Neu5Ac) and G.sub.M.sub.3(Neu5Gc). A slightly enhanced activity, compared with reference Vibrio cholerae sialidase, was detected towards terminally α(2-3)-sialylated neolacto-series gangliosides IV.sup.3-α- Neu5Ac-nLc.sub.4Cer and VI.sup.3-α- Neu5Ac-nLc.sub.6Cer. The ganglio-series gangliosides G.sub.D.sub.1.sub.a, G.sub.D.sub.1.sub.b, and G.sub.T.sub.1.sub.b, the preferential substrates of V. cholerae sialidase for generating cleavage-resistant G.sub.M.sub.1, were less suitable targets for the CHO cell sialidase. The increasing evidence on colocalization of gangliosides and sialidase in the cytosol strongly suggests the involvement of the cytosolic sialidase in ganglioside metabolism on intracellular level by yet unknown mechanisms. .COPYRGT. 2001 Elsevier Science Ltd.

```
=> Neu5Ac(10A)Neu5Gc
L57          8 FILE AGRICOLA
L58         36 FILE BIOTECHNO
L59          0 FILE CONFSCI
L60          0 FILE HEALSAFE
L61         21 FILE LIFESCI
L62         12 FILE PASCAL

TOTAL FOR ALL FILES
L63         77 NEU5AC(10A) NEU5GC

=> l63 and antibody
L64          1 FILE AGRICOLA
L65          6 FILE BIOTECHNO
L66          0 FILE CONFSCI
L67          0 FILE HEALSAFE
L68          6 FILE LIFESCI
L69          5 FILE PASCAL

TOTAL FOR ALL FILES
L70         18 L63 AND ANTIBODY

=> dup rem
ENTER L# LIST OR (END):l70
PROCESSING COMPLETED FOR L70
L71          10 DUP REM L70 (8 DUPLICATES REMOVED)

=> d l71 ibib abs total

L71  ANSWER 1 OF 10  AGRICOLA  Compiled and distributed by the National
     Agricultural Library of the Department of Agriculture of the United States
     of America.  It contains copyrighted materials.  All rights reserved.
     (2009) on STN                                        DUPLICATE 1
ACCESSION NUMBER:        2008:127164  AGRICOLA
DOCUMENT NUMBER:         IND44106990
TITLE:                   Diversity in specificity, abundance, and composition
                         of anti-Neu5Gc antibodies in normal humans:
                         Potential implications for disease.
AUTHOR(S):               Padler-Karavani, Vered; Yu, Hai; Cao, Hongzhi;
                         Chokhawala, Harshal; Karp, Felix; Varki, Nissi; Chen,
                         Xi; Varki, Ajit
SOURCE:                  Glycobiology, 2008 Oct. Vol. 18, no. 10 p. 818-830
                         ISSN: 0959-6658
NOTE:                    Includes references
DOCUMENT TYPE:           Article
FILE SEGMENT:            Non-US
```

LANGUAGE: English

AB Human heterophile antibodies that agglutinate animal erythrocytes are known to detect the nonhuman sialic acid N-glycolylneuraminic acid (Neu5Gc). This monosaccharide cannot by itself fill the binding site (paratope) of an antibody and can also be modified and presented in various linkages, on diverse underlying glycans. Thus, we hypothesized that the human anti-Neu5Gc antibody response is diverse and polyclonal. Here, we use a novel set of natural and chemoenzymatically synthesized glycans to show that normal humans have an abundant and diverse spectrum of such anti-Neu5Gc antibodies, directed against a variety of Neu5Gc-containing epitopes. High sensitivity and specificity assays were achieved by using N-acetylneuraminic acid (Neu5Ac)-containing probes (differing from Neu5Gc by one less oxygen atom) as optimal background controls. The commonest anti-Neu5Gc antibodies are of the IgG class. Moreover, the range of reactivity and Ig classes of antibodies vary greatly amongst normal humans, with some individuals having remarkably large amounts, even surpassing levels of some well-known natural blood group and xenoreactive antibodies. We purified these anti-Neu5Gc antibodies from individual human sera using a newly developed affinity method and showed that they bind to wild-type but not Neu5Gc-deficient mouse tissues. Moreover, they bind back to human carcinomas that have accumulated Neu5Gc in vivo. As dietary Neu5Gc is primarily found in red meat and milk products, we suggest that this ongoing antigen-antibody reaction may generate chronic inflammation, possibly contributing to the high frequency of diet-related carcinomas and other diseases in humans.

L71 ANSWER 2 OF 10 LIFESCI COPYRIGHT 2009 CSA on STN

ACCESSION NUMBER: 2007:154349 LIFESCI

TITLE: Germinal Center Marker GL7 Probes Activation-Dependent Repression of N-Glycolylneuraminic Acid, a Sialic Acid Species Involved in the Negative Modulation of B-Cell Activation

AUTHOR: Naito, Yuko; Takematsu, Hiromu; Koyama, Susumu; Miyake, Shizu; Yamamoto, Harumi; Fujinawa, Reiko; Sugai, Manabu; Okuno, Yasushi; Tsujimoto, Gozoh; Yamaji, Toshiyuki; Hashimoto, Yasuhiro; Itohara, Shigeyoshi; Kawasaki, Toshisuke; Suzuki, Akemi; Kozutsumi, Yasunori

CORPORATE SOURCE: Laboratory of Membrane Biochemistry and Biophysics, Graduate School of Biostudies. Department of Biological Chemistry. Department of Genomic Drug Discovery, Graduate School of Pharmaceutical Sciences. Center for Genomic Medicine, Graduate School of Medicine, Kyoto University, Sakyo, Kyoto 606-8501, Japan. Supra-Biomolecular System Research Group, RIKEN Frontier Research System. Laboratory for Behavioral Genetics, RIKEN Brain Science Institute, RIKEN, Wako, Saitama 351-0198, Japan. CREST, Japan Science and Technology, Kawaguchi, Saitama, Japan

SOURCE: Molecular and Cellular Biology [Mol. Cell. Biol.], (20070400) vol. 27, no. 8, pp. 3008-3022. ISSN: 0270-7306.

DOCUMENT TYPE: Journal

FILE SEGMENT: N

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Sialic acid (Sia) is a family of acidic nine-carbon sugars that occupies the nonreducing terminus of glycan chains. Diversity of Sia is achieved by variation in the linkage to the underlying sugar and modification of the Sia molecule. Here we identified Sia-dependent epitope specificity for GL7, a rat monoclonal antibody, to probe germinal centers upon T cell-dependent immunity. GL7 recognizes sialylated glycan(s), the alpha

2,6-linked N-acetylneuraminic acid (Neu5Ac) on a lactosamine glycan chain(s), in both Sia modification- and Sia linkage-dependent manners. In mouse germinal center B cells, the expression of the GL7 epitope was upregulated due to the in situ repression of CMP-Neu5Ac hydroxylase (Cmah), the enzyme responsible for Sia modification of Neu5Ac to Neu5Gc. Such Cmah repression caused activation-dependent dynamic reduction of CD22 ligand expression without losing alpha 2,6-linked sialylation in germinal centers. The in vivo function of Cmah was analyzed using gene-disrupted mice. Phenotypic analyses showed that Neu5Gc glycan functions as a negative regulator for B-cell activation in assays of T-cell-independent immunization response and splenic B-cell proliferation. Thus, Neu5Gc is required for optimal negative regulation, and the reaction is specifically suppressed in activated B cells, i.e., germinal center B cells.

L71 ANSWER 3 OF 10 PASCAL
on STN

ACCESSION NUMBER: 2007-0300542 PASCAL
TITLE (IN ENGLISH): Role of sialic acid in bovine sperm-zona pellucida binding
AUTHOR: GUILLERMO VELASQUEZ Jose; CANOVAS Sebastian; BARAJAS Patricia; MARCOS Julian; JIMENEZ-MOVILLA Maria; GUTIERREZ GALLEGO Ricardo; BALLESTA Jose; AVILES Manuel; COY Pilar
CORPORATE SOURCE: Department of Physiology, Veterinary Faculty, University ofMurcia, Murcia, Spain; Colombian Corporation for Agricultural Research and University of La Salle, Villavicencio, Meta, Colombia; Department of Cell Biology, Medicine Faculty, University ofMurcia, Murcia, Spain; Pharmacology Research Unit, Municipal Institute of Medical Research, Department of Experimental and Health Sciences, University Pompeu Fabra, Barcelona, Spain
SOURCE: Molecular reproduction and development : (Print), (2007), 74(5), 617-628, refs. 1 p.1/2
ISSN: 1040-452X CODEN: MREDEE
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-18057, 354000159363630110

AN 2007-0300542 PASCAL
AB Sperm binding activity has been detected in zona pellucida (ZP) glycoproteins and it is generally accepted that this activity resides in the carbohydrate moieties. In the present study we aim to identify some of the specific carbohydrate molecules involved in the bovine sperm-ZP interaction. We performed sperm binding competition assays, in vitro fecundation (IVF) in combination with different lectins, antibodies and neuraminidase digestion, and chemical and cytochemical analysis of the bovine ZP. Both MAA lectin recognising α-2,3-linked sialic acid and neuraminidase from Salmonella typhimurium with catalytic activity for α-2,3-linked sialic acid, demonstrated a high inhibitory effect on the sperm-ZP binding and oocyte penetration. These results suggest that bovine sperm-ZP binding is mediated by α-2,3-linked sialic acid. Experiments with trisaccharides (sialyllactose, 3'-sialyllactosamine and 6'-sialyllactosamine) and glycoproteins (fetuin and asialofetuin) corroborated this and suggest that at least the sequence Neu5A-c(α2-3)Gal(β1-4)GlcNAc is involved in the sperm-ZP interaction. Moreover, these results indicate the presence of a sperm plasma membrane specific protein for the sialic acid. Chemical analysis

revealed that bovine ZP glycoproteins contain mainly Neu5Ac (84.5%) and Neu5GC (15.5%). These two types of sialic acid residues are probably linked to Galβ1,4Glc-NAc and GalNAc by α-2,3- and α-2,6-linkages, respectively, as demonstrated by lectin cytochemical analysis. The use of a neuraminidase inhibitor resulted in an increased number of spermatozoa bound to the ZP and penetrating the oocyte. From this last result we hypothesize that a neuraminidase from cortical granules would probably participate in the block to polyspermy by removing sialic acid from the ZP.

L71 ANSWER 4 OF 10 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN DUPLICATE

ACCESSION NUMBER: 2003:36818433 BIOTECHNO
TITLE: Effects of buffering conditions and culture pH on production rates and glycosylation of clinical phase I anti-melanoma mouse IgG3 monoclonal antibody R24
AUTHOR: Muthing J.; Kemminer S.E.; Conradt H.S.; Sagi D.; Nimtz M.; Karst U.; Peter-Katalinic J.
CORPORATE SOURCE: Dr. J. Muthing, Inst. for Med. Phys. and Biophysics, Laboratory for Biomedical Analysis, University of Munster, D-48149 Munster, Germany. E-mail: jm@uni-muenster.de
SOURCE: Biotechnology and Bioengineering, (05 AUG 2003), 83/3 (321-334), 88 reference(s) CODEN: BIBIAU ISSN: 0006-3592
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English

AN 2003:36818433 BIOTECHNO

AB R24, a mouse IgG3 monoclonal antibody (MAb) against ganglioside GD3 (Neu5Acα8Neu5Acα3Gal β4Glcβ1Cer), can block tumor growth as reported in a series of clinical trials in patients with metastatic melanoma. The IgG molecule basically contains an asparagine-linked biantennary complex type oligosaccharide on the C.sub.H2 domain of each heavy chain, which is necessary for its in vivo effector function. The purpose of this study was to investigate the biotechnological production and particularly the glycosylation of this clinically important MAb in CO.sub.2/HCO.sub.3.sup.- (pH 7.4, 7.2, and 6.9) and HEPES buffered serum-free medium. Growth, metabolism, and IgG production of hybridoma cells (ATCC HB-8445) were analyzed on a 2-L bioreactor scale using fed-batch mode. Specific growth rates (μ) and MAb production rates (q.sub.I.sub.g.sub.G) varied significantly with maximum product yields at pH 6.9 (q.sub.I.sub.g.sub.G = 42.9 μg 10.sup.-.sup.6 cells d.sup.-.sup.1, p = 0.30 d.sup.-.sup.1) and lowest yields in pH 7.4 adjusted batches (q.sub.I.sub.g.sub.G = 10.8 μg 10.sup.-.sup.6 cells d.sup.-.sup.1, μ = 0.40 d.sup.-.sup.1). N-glycans were structurally characterized by high pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), and electrospray-ionization quadrupole time-of-flight (ESI-QTOF) mass spectrometry (MS). The highest relative amounts of agalacto and monogalacto biantennary complex type oligosaccharides were detected in the pH 7.2 (46% and 38%, respectively) and pH 6.9 (44% and 40%, respectively) cultivations and the uppermost quantities of digalacto (fully galactosylated) structures in the pH 7.4 (32%) and the HEPES (26%) buffered fermentation. In the experiments with HEPES buffering, antibodies with a molar Neu5Ac/Neu5Gc ratio of 3.067 were obtained. The fermentations at pH 7.2 and 6.9 resulted in almost equal molar Neu5Ac/Neu5Gc ratios of 1.008 and

0.985, respectively, while the alkaline shift caused a moderate overexpression of Neu5Ac deduced from the Neu5Ac/Neu5Gc quotient of 1.411. Different culture buffering gave rise to altered glycosylation pattern of the MAb R24. Consequently, a detailed molecular characterization of MAb glycosylation is generally recommended as a part of the development of MAbs for targeted in vivo immunotherapy to assure biochemical consistency of product lots and oligosaccharide-dependent biological activity. .COPYRGT. 2003 Wiley Periodicals, Inc.

L71 ANSWER 5 OF 10 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 2002:34451176 BIOTECHNO
TITLE: Ganglioside expression in tissues of mice lacking β.sub.2-microglobulin
AUTHOR: Markotic A.; Marusic A.; Tomac J.; Muthing J.
CORPORATE SOURCE: A. Markotic, Split University School of Medicine, Soltanska 2, 21000 Split, Croatia.
E-mail: markotic@bsb.mefst.hr
SOURCE: Clinical and Experimental Immunology, (2002), 128/1 (27-35), 65 reference(s)
CODEN: CEXIAL ISSN: 0009-9104
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2002:34451176 BIOTECHNO
AB This study presents a comparative analysis of gangliosides from lymphoid (spleen and thymus) and other (brain, liver, lungs and muscle) tissues of C57BL/6 mice lacking the gene for β.sub.2-microglobulin (β.sub.2M), a constitutive component of the MHC class I molecule. Ganglioside fractions in the tissues of mice homozygous (β.sub.2M-/-) and heterozygous (β.sub.2M-/+) for the gene deletion were determined by high performance thin-layer chromatography (HPTLC), followed by immunostaining with specific polyclonal antibodies. Ubiquitous gangliosides G.sub.M.sub.3(Neu5Ac) and G.sub.M.sub.3(Neu5Gc) were the dominant gangliosides in the lungs of the control β.sub.2M-/+ mice, whereas the homozygous knockout mice had substantially decreased expression of these structures. The lungs of the β.sub.2M-/- mice also had reduced expression of T-lymphocyte-specific G.sub.M.sub.1.sub.b-type gangliosides (G.sub.M.sub.1.sub.b and GalNAc-G.sub.M.sub.1.sub.b). β.sub.2M-deficient mice also had more G.sub.M.sub.1.sub.a and G.sub.D.sub.1.sub.a gangliosides in the liver, and several neolacto-series gangliosides were increased in the brain and lungs. This study provides in vivo evidence that the β.sub.2M molecule can influence the acquisition of a distinct ganglioside assembly in different mouse organs, implicating its non-immunological functions.

L71 ANSWER 6 OF 10 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 2001:32972641 BIOTECHNO
TITLE: Production and molecular characterization of clinical phase I anti-melanoma mouse igG3 monoclonal antibody R24
AUTHOR: Kemminer S.E.; Conradt H.S.; Nimtz M.; Sagi D.; Peter-Katalinic J.; Diekmann O.; Drmic I.; Muthing J.
CORPORATE SOURCE: J. Muthing, Institute of Cell Culture Technology, University of Bielefeld, P.O.Box 100131, 33501 Bielefeld, Germany.
E-mail: jm@zellkult.techfak.uni-bielefeld.de

SOURCE: Biotechnology Progress, (2001), 17/5 (809-821), 85 reference(s)
CODEN: BIPRET ISSN: 8756-7938
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2001:32972641 BIOTECHNO
AB R24 is a mouse IgG3 monoclonal antibody (mab) that reacts with the ganglioside GD3 expressed by cells of neuroectodermal origin. The anti-tumor activity of R24 has been demonstrated in initial phase I and pilot trials in patients suffering from metastatic melanoma. The purpose of this study was to investigate the biotechnological production and particularly the glycosylation of this clinically important antibody. Growth, metabolism, and IgG production of R24 secreting hybridoma cells were analyzed on 1 L bioreactor bench scale using repeated-batch mode. The amount of 57 mg of pure mab was obtained from 1.6 L crude supernatant by protein A chromatography. Western blot binding assays with sugar-specific lectins revealed glycosylation of the heavy chains, whereas no carbohydrates were detectable on the light chains. Because glycosylation is essential for antibody effector functions in vivo (such as complement fixation or binding to macrophage Fc receptors), mab R24 was subjected to both enzymatic deglycosylation using PNGase F and chemical deglycosylation by hydrazinolysis. Released glycans were structurally characterized by high pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), matrix assisted laser desorption ionization time-of-flight (MALDI-TOF), and electrospray ionization quadrupole time-of-flight (ESI-QTOF) mass spectrometry. Six major biantennary chains of the complex glycosylation phenotype were found with variations in galactosylation and core fucosylation. The predominant N-linked structure, indicating the high degree of agalactosyl glycoforms, was the agalacto biantennary chain with a relative percentage of 57% (51% core-fucosylated, 6% nonfucosylated). The second most abundant oligosaccharide was the monogalacto biantennary chain amounting to 30% (26% core- and 4% nonfucosylated). The antibody contained 0.46 μg sialic acid per mg protein, which splits into 0.243 μg Neu5Gc and 0.217 μg Neu5Ac , corresponding to a Neu5Ac:Neu5Gc ratio of 1:1.06. Furthermore, the antigen specificity of R24 was determined by immunodetection of GD3 on thin-layer chromatograms, and real time GD3-antibody binding interactions were measured with an optical biosensor (BIAcore). From the structural data obtained in this study it is concluded that glycosylation of the antibody may be important in the clinical outcome of targeted anti-cancer immunotherapy.

L71 ANSWER 7 OF 10 BIOTECHNO
ACCESSION NUMBER: 2001:32206403 BIOTECHNO
TITLE: Characterization of cytosolic sialidase from Chinese hamster ovary cells - Part II. Substrate specificity for gangliosides
AUTHOR: Muthing J.; Burg M.
CORPORATE SOURCE: J. Muthing, Institute of Cell Culture Technology, Faculty of Technology, University of Bielefeld, PO Box 100131, D-33501 Bielefeld, Germany.
E-mail: jm@zellkult.techfak.uni-bielefeld.de
SOURCE: Carbohydrate Research, (15 FEB 2001), 330/3 (347-356), 58 reference(s)
CODEN: CRBRAT ISSN: 0008-6215
PUBLISHER ITEM IDENT.: S0008621500002950
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom

LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2001:32206403 BIOTECHNO
AB Cytosolic Chinese hamster ovary (CHO) cell sialidase has been cloned as a soluble glutathione S-transferase (GST)-sialidase fusion protein with an apparent molecular weight of 69 kD in Escherichia coli. The enzyme has then been produced in mg quantities at 25-L bioreactor scale and purified by one-step affinity chromatography on glutathione sepharose (Burg, M.; Muthing, J. Carbohydr. Res. 2001, 330, 335-346). The cloned sialidase was probed for desialylation of a wide spectrum of different types of gangliosides using a thin-layer chromatography (TLC) overlay kinetic assay. Different gangliosides were separated on silica gel precoated TLC plates, incubated with increasing concentrations of sialidase (50 μU/mL up to 1.6 mU/mL) without detergents, and desialylated gangliosides were detected with specific anti-asialoganglioside antibodies. The enzyme exhibited almost identical hydrolysis activity in degradation of G.sub.M.sub.3(Neu5Ac) and G.sub.M.sub.3(Neu5Gc). A slightly enhanced activity, compared with reference Vibrio cholerae sialidase, was detected towards terminally α(2-3)-sialylated neolacto-series gangliosides IV.sup.3-α-Neu5Ac-nLc.sub.4Cer and VI.sup.3-α-Neu5Ac-nLc.sub.6Cer. The ganglio-series gangliosides G.sub.D.sub.1.sub.a, G.sub.D.sub.1.sub.b, and G.sub.T.sub.1.sub.b, the preferential substrates of V. cholerae sialidase for generating cleavage-resistant G.sub.M.sub.1, were less suitable targets for the CHO cell sialidase. The increasing evidence on colocalization of gangliosides and sialidase in the cytosol strongly suggests the involvement of the cytosolic sialidase in ganglioside metabolism on intracellular level by yet unknown mechanisms. .COPYRGT. 2001 Elsevier Science Ltd.

L71 ANSWER 8 OF 10 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1998:28318131 BIOTECHNO
TITLE: Large scale purification of gangliosides G(M3)(Neu5Ac) and G(M3)(Neu5Gc) by trimethylaminoethyl-fractogel high-performance liquid chromatography
AUTHOR: Heitmann D.; Ziehr H.; Muthing J.
CORPORATE SOURCE: J. Muthing, Institut fur Zellkulturtechnik, Universitat Bielefeld, P.O. Box 10 01 31, 33501 Bielefeld, Germany.
SOURCE: Journal of Chromatography B: Biomedical Applications, (12 JUN 1998), 710/1-2 (1-8), 38 reference(s)
CODEN: JCBBEP ISSN: 0378-4347
PUBLISHER ITEM IDENT.: S0378434798001224
DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28318131 BIOTECHNO
AB A preparative anion-exchange high-performance liquid chromatographic method for the separation of the closely allied monosialogangliosides G(M3)(Neu5Ac) and G(M3)(Neu5Gc) has been developed. Hybridoma cells, readily available material derived from industrial monoclonal antibody production, were used as ganglioside source and led to fractions with pure G(M3)(Neu5Ac) and G(M3)(Neu5Gc) in high milligram quantities. The crude ganglioside extract was loaded onto columns filled with the strong anion-exchanger trimethylaminoethyl (TMAE)-Fractogel. Gangliosides were eluted from the stationary phase with a gradient system of ammonium acetate in methanol. The scaled-up approach ranged over more than one order of magnitude from

20 to 500 mg batches of G(M3) gangliosides. Thus, the high-resolution power of the strong anion-exchanger TMAE-Fractogel allowed the preparative isolation by one-step column chromatography of two G(M3) specimens which only differ in one hydroxyl group at position 5 of the neuraminic acid (N-acetyl- versus N- glycolylneuraminic acid).

L71 ANSWER 9 OF 10 BIOTECHNO COPYRIGHT 2009 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1995:25217944 BIOTECHNO
TITLE: Variation in N-linked carbohydrate chains in different batches of two chimeric monoclonal IgG1 antibodies produced by different murine SP2/0 transfectoma cell subclones
AUTHOR: Bergwerff A.A.; Stroop C.J.M.; Murray B.; Holtorf A.-P.; Pluschke G.; Van Oostrum J.; Kamerling J.P.; Vliegenthart J.F.G.
CORPORATE SOURCE: Department of Bio-Organic Chemistry, Bijvoet Center, Utrecht University, P O Box 80.075,NL-3508 TB Utrecht, Netherlands.
SOURCE: Glycoconjugate Journal, (1995), 12/3 (318-330)
CODEN: GLJOEW ISSN: 0282-0080
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1995:25217944 BIOTECHNO
AB Two chimeric human/murine monoclonal antibodies were constructed by substitution of the murine constant regions with human γ1 and κ constant regions for heavy and light chains, respectively. The chimeric human/murine molecules are anti-idiotypic antibodies, meaning that they were directed against the antigen binding site in the variable region of another antibody. Antibody batches were produced under identical production conditions, using two selected SP2/0 myeloma cell subclones, which produce chimeric antibodies with different variable regions, but identical constant regions. Several samples were collected during the production of the antibodies in hollow- fibre reactors. The heavy chain, but not the light chain, of the two different chimeric IgG1 antibodies is glycosylated. Structural analysis of the enzymically released N-linked carbohydrate chains by .sup.1H-NMR spectroscopy, as well as by chromatographic profiling, demonstrated that the collection of N-glycans comprises a small amount of monoantennary, and for the greater part diantennary structures. The N-glycans are completely (α1 → 6)-fucosylated at the innermost GlcNAc residue. The antennae of the neutral diantennary N-glycans are built up from GlcNAcβ1 → 2, Galβ1 → 4GlcNAcβ1 → 2 or Galα1 → 3Gα1β1 → 4GlcNAcβ1 → 2 elements, whereas the antennae of the neutral monoantennary carbohydrate chains have only (β1 → 2)-linked GlcNAc residues. Galactosylation of the GlcNAcβ1 → 2Manα1 → 6 branch occurs four times more frequently than that of the GlcNAcβ1 → 2Manα1 → 3 branch, independently of the production batch. A small amount of the diantennary N-glycans are mono- or disialylated, carrying N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc), exclusively (α2 → 6)- linked to βGal. Analysis of the different production batches demonstrates that the structures of the N-linked carbohydrate chains are identical in the two chimeric antibodies, but that the relative amounts of the major oligosaccharide components, the degree of sialylation and the molar ratio of Neu5Ac to Neu5Gc varies with the SP2/0 cell subclone, and only slightly with cell age.

L71 ANSWER 10 OF 10 LIFESCI COPYRIGHT 2009 CSA on STN
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TITLE: Use of a bacteriophage-derived endo-N-acetylneuraminidase and an equine antipolysialyl antibody to characterize the polysialyl residues in salmonid fish egg polysialoglycoproteins. Substrate and immunospecificity studies.
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SUMMARY LANGUAGE: English
AB Polysialoglycoproteins (PSGP), a class of glycoproteins containing oligo(poly)sialylglycan chains, are the major glycoprotein components in cortical alveoli of a number of Salmonidae fish eggs. Lake trout, Salvelinus namaycush , egg PSGP (PSGP(Sn) differs from rainbow trout, Salmo gairdneri , egg PSGP (PSGP(Sg)) in its sialic acid composition. Fragmentation analysis of oligo(poly)sialyl chains in lake trout PSGP(Sn) has established that there are two distinct types of oligo(poly)sialyl structures in this PSGP molecule, namely alpha -2,8-linked oligo/poly(Neu5Ac) and alpha -2,8-linked oligo/poly(Neu5Gc). Endo-N-acetylneuraminidase was found to catalyze the hydrolysis of both alpha -2,8-linked poly (Neu5Ac) and poly(Neu5Gc), so that this enzyme can be used as a diagnostic reagent for detecting both types of polysialic acids. H.46 was used in indirect immunofluorescence experiments to localize PSGP(Sn) in cortical alveoli isolated from lake trout eggs.